PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	18 May 2005 (18-05-2005)
Applicant's or agent's file reference 753-118PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA2005/000069	24 January 2005 (24-01-2005)	23 January 2004 (23-01-2004)

International Patent Classification (IPC) or both national classification and IPC
IPC7:A61K 48/00; A61P 35/00; A61K 31/7088; A61K 31/522; A61K 31/517; A61K 31/513

Applicant
SARISSA INC. ET AL

1. This opinion contains indications relating to the following items :

[X]	Box No. I	Basis of the opinion
[ ]	Box No. II	Priority
[X]	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
[X]	Box No. IV	Lack of unity of invention
[X]	Box No. V	Reasoned statement under Rule 43*bis*.1(a)(I) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
[ ]	Box No. VI	Certain documents cited
[X]	Box No. VII	Certain defects in the international application
[X]	Box No. VIII	Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

Name and mailing address of the ISA/CA	Date of completion of this opinion	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street	22 April 2005 (22.04.2005)	Debora Fujimoto (819) 997-1855

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Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   [ ] the international application in the language in which it was filed

   [ ] a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of :

   a. type of material

      [X] a sequence listing

      [ ] table(s) related to the sequence listing

   b. format of material

      [X] on paper

      [X] in electronic form

   c. time of filing/furnishing

      [X] contained in the international application as filed.

      [X] filed together with the international application in electronic form

      [ ] furnished subsequently to this Authority for the purposes of search.

3 [X] In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statement that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments :

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of :

[ ] the entire international application

[ ] claim Nos.

because:

[X] the said international application, or the said claim Nos. 1 to 26 relate to the following subject matter which does not require an international search *(specify)* :

Although claims 1 to 26 encompass a method of treatment of the human/animal body which this Authority is not required to examine under Rule 67.1(iv) of the PCT, the written opinion has been established on the basis of the alleged effects of the compounds referred to therein.

[ ] the description, claims or drawings *(indicate particular elements below)* or said claim Nos. are so unclear that no meaningful opinion could be formed *(specify)* :

[ ] the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify):*

[ ] no international search report has been established for said claims Nos.

[ ] a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

[ ] furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

[ ] furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

[ ] pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

[ ] a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.

[ ] the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

[ ] See Supplemental Box for further details.

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Box No. IV Lack of unity of invention

1. [ ] In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit :

   [ ] paid additional fees

   [ ] paid additional fees under protest and, where applicable, the protest fee

   [ ] paid additional fees under protest but the applicable protest fee was not paid

   [ ] not paid additional fees

2. [ ] This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   [ ] complied with

   [ ] not complied with for the following reasons :

4: Consequently, this opinion has been established in respect of the following parts of the international application :

   [X] all parts

   [ ] the parts relating to claim Nos.

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(I) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-17, 25, 26	YES
	Claims	18-24	NO
Inventive step (IS)	Claims		YES
	Claims	1-26	NO
Industrial applicability (IA)	Claims	1-26 (partially)	YES
	Claims	1-26 (partially)	NO

2. Citations and explanations :

D1 WO 9963114 A1 (ISIS PHARMACEUTICALS, INC.) 09.12.1999
D2 CA 2301957 A1 (ZENECA LIMITED, ISIS PHARMACEUTICALS) 01.04.1999
D3 FERGUSON PJ et al. Antisense down-regulation of thymidylate synthase to suppress growth and enhance cytotoxicity of 5-FUdR, 5-FU and Tomudex in HeLa cells. BRIT J PHARMACOL 1999 Vol 127, pp 1777-1786
D4 BERG RW et al. The means to an end of tumor cell resistance to chemotherapeutic drugs targeting thymidylate synthase: shoot the messenger. CURR DRUG TARGETS 2002 Vol 3, pp 297-309
D5 BERG RW et al. Tumor growth inhibition *in vivo* and G_2/M cell cycle arrest induced by antisense oligodeoxynucleotide targeting thymidylate synthase. J PHARMACOL EXP THER 2001 Vol 298(2), pp 477-484
D6 FERGUSON PJ et al. Antisense-induced down-regulation of thymidylate synthase and enhanced cytotoxicity of 5-FUdR in 5-FUdR-resistant HeLa cells. BRIT J PHARMACOL 2001 Vol 134, pp 1437-1446
D7 SCHMITZ JC et al. Effect of 2'-*O*-methyl antisense ORNs on expression of thymidylate synthase in human colon cancer RKO cells. NUCLEIC ACIDS RES 2001 Vol 29(2), pp 415-422

The problem to be solved is the treatment of a neoplastic condition, such as mesothelioma, with an antisense oligonucleotide (ODN) complementary to a thymidylate synthase (TS) mRNA used alone, or in combination with a chemotherapeutic agent, wherein said antisense ODN enhances the cytotoxic effect of the chemotherapeutic agent.

D1, D2, D3, or D4 disclose the use of antisense oligonucleotides (ODNs) containing 2'-methoxyethoxy and phosphorothioate modifications, complementary to the sequence of thymidylate synthase, to inhibit proliferation of neoplastic cells *in vitro*. D1 discloses that the 20-mer depicted in SEQ ID NO:4, which is identical to SEQ ID NO:1 (5'-GCCAGTGGCAACATCCTTAA-3') of the present application, used alone (Example 2) or in combination with a chemotherapeutic agent, Tomudex™ (raltitrexed; Example 3), inhibits proliferation of HeLa cells. D2, D3, or D4 disclose that ODN 83, which is identical to SEQ ID NO:1 of the present application, used alone or in combination with a chemotherapeutic agent, inhibits the proliferation of neoplastic cells. Thus, any one of D1-D4 disclose the use of the antisense ODN that is identical to SEQ ID NO:1 of the present application to treat neoplastic cells *in vitro*.

D2 further demonstrates that the use of ODN 83 sensitizes HeLa cells to the cytotoxic effects of 5-FU, 5-FUdR, Tomudex™ (raltitrexed), and methotrexate (MTX), but not to cisplatin or chloroambucil (Fig. 13). Additionally, D3 discloses that the use of antisense ODNs targeted to regions in the TS sequence other than that targeted by ODN 83, alone or in combination with the chemotherapeutic agent, Tomudex™, are either ineffective or enhance growth and survival in cancer cells (page 22, line 22 to page 23, line 2; Fig. 2). Thus, the specific sequence of the antisense ODN determines its utility for the inhibition of neoplastic cells.

(Continued in Supplemental Box)

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Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted :

In claim 18, the following typographical error has been noted: "cytotoxity".

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made :

Claims 1, 2, 4-11, 13-20, and 22-26 do not comply with Article 6 of the PCT. Claims 1, 2, 4-11, and 13-20, and 22-26, directed to the use of any antisense oligonucleotide (ODN) of 7 to about 100 nucleotides in length, comprising a sequence of at least 7 consecutive nucleotides that is complementary to a thymidylate synthase mRNA, are not fully supported in the description. In the present application, Applicant has only disclosed the use of the 20-mer antisense ODN depicted in SEQ ID NO:1.

Claims 16 and 26 do not comply with Article 6 of the PCT. It is unclear if the mesothelioma cells are resistant to the specific chemotherapeutic drug that is used in the combination therapy, or if said cells are resistant to any chemotherapeutic drug, in general.

Claims 9 and 18 do not comply with Article 6 of the PCT. In claims 9 and 18, the phrase "effective amount" lacks clarity. In claim 18, the phrase "enhancing the cytotoxicity of a chemotherapeutic agent" lacks clarity, as it is unclear if the "enhancing" effect is compared to the use of the antisense ODN alone or to the use of the chemotherapeutic agent alone. Further, in claim 18, it is unclear if "a chemotherapeutic agent" used in combination with the antisense ODN is the same chemotherapeutic agent that has enhanced cytotoxicity through the use of the method.

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: **Box V.** (continued)

D3 further discloses that the use of ODN 83 down-regulates TS and results in inhibition of HeLa cell proliferation and enhancement of the cytotoxic effects of 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), and Tomudex™ (raltitrexed), that are all known to target TS, but not of cisplatin or chlorambucil, which do not target TS (Fig. 5; Table 1).

D4 further discloses that the sensitivity of ODN 83-treated cells to the cytotoxic effects of specific TS-targeting chemotherapeutic drugs (5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), and raltitrexed) is increased, but the sensitivity to non-TS-targeting drugs (cisplatin, melphalan, doxorubicin, and paclitaxel; Table 1) is not. Additionally, D4 discloses the use of the combination of ODN 83 and a chemotherapeutic drug enhanced the *in vivo* cytotoxicity of said drug to a colon tumor xenograft in a mouse, when compared to the use of the TS antisense ODN 83 alone. D4 reviews examples of antisense ODNs targeted other genes, e.g., c-raf, NER2/neu, or protein kinase Cα, to sensitize cancer cell lines to the cytotoxic effects of a chemotherapeutic drug (page 303, second column, second paragraph).

D5 discloses that the use of ODN 83, identical to SEQ ID NO:1 of the present application, inhibited human colon cancer cells in vivo. Additionally, the use of ODN 83 resulted in the inhibition of cell proliferation and sensitization of HeLa cells or human colon cancer cells to TS-targeting chemotherapeutic drugs *in vitro*, as compared to the treatment of cells with 5-FU or raltitrexed alone.

D6 discloses that ODN 83, identical to SEQ ID NO:1 of the present application, down-regulated TS protein and enhanced cytotoxicity of the TS-targeting drug, 5-FUdR, in 5-FUdR-resistant HeLa cells.

D7 discloses that a 30-mer antisense 2'-*O*-methyl oligoribonucleotide (ORN) and an 18-mer antisense ORN targeting the same 5' upstream target region on TS mRNA (nucleotides 80-109 of TS mRNA) repressed TS expression in human colon cancer cells, but that an ORN smaller than an 18-mer did not inhibit the expression of TS protein in human colon cancer cells. D7 also discloses that human cancer can be treated with the use of an antisense ORN alone or in combination with other anti-cancer agents.

Novelty:
D1, D2, or D3 disclose methods using the combination of the antisense ODN that is depicted in SEQ ID NO:1 of the present application and a chemotherapeutic drug to enhance the cytotoxicity of said drug to neoplastic cells *in vitro*. Accordingly, claims 18-23 are considered to lack novelty in view of any one of D1-D3, and therefore, are not compliant with Article 33(2) of the PCT.

D4 discloses the *in vivo* use of the antisense ODN that is depicted in SEQ ID NO:1 of the present application and a chemotherapeutic drug to enhance the cytotoxicity of the drug to a colon tumor xenograft in a mouse. Accordingly, claims 18-24 are considered to lack novelty in view of D4, and therefore, are not compliant with Article 33(2) of the PCT.

Claims 18-24 are considered to lack novelty under Article 33(2) of the PCT. Claims 1-17, 25, and 26 appear to satisfy the requirements of Article 33(2) of the PCT.

Inventive Step:
In view of D1-D4, discussed above, claims 18-24 also lack an inventive step under Article 33(3) of the PCT.

D1, D2, D3, or D4 disclose the use of the antisense ODN that is depicted in SEQ ID NO:1 of the present application, alone or in combination with a chemotherapeutic drug, to inhibit a variety of human neoplastic cells. D2 additionally discloses the treatment of neoplastic cells *in vivo* with said antisense ODN alone or in combination with a chemotherapeutic drug. D6 additionally discloses the use of said antisense ODN to enhance cytotoxicity in drug-resistant neoplastic cells. Thus, it is obvious to one of skill in the art to use said antisense ODN alone or in combination with a chemotherapeutic drug for the treatment of a specific cancer, mesothelioma. Accordingly, claims 1-17, 25, and 26 lack an inventive step in view of any one of D1-D4 taken together with D6, and therefore, are not compliant with Article 33(3) of the PCT.

(Continued in Supplemental Box, page 2)

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: **Box V.** (page 2)

D5 discloses the use of the antisense ODN that is depicted in SEQ ID NO:1 of the present application, to inhibit human colon cancer cells *in vivo*. D5 further reviews examples of the use of an antisense ODN to a target gene and a chemotherapeutic drug to inhibit different neoplastic cells. D6 discloses the use of the antisense ODN that is depicted in SEQ ID NO:1 of the present application to enhance cytotoxicity in 5-FUdR-resistant HeLa cells to the TS-targeting drug, 5-FUdR. D7 discloses the use of antisense ORNs of various lengths, targeted to TS, to inhibit human colon cancer cells. Thus, in view of D5, D6, and D7, it is obvious to one of skill in the art to use an antisense ODN targeted to thymidylate synthase alone or in combination with a chemotherapeutic drug to enhance cytotoxic effects and specifically inhibit both drug-sensitive and drug-resistant mesothelioma cells. Accordingly, claims 1-17, 25, and 26 lack an inventive step in view of D5 to D7, and therefore, are not compliant with Article 33(3) of the PCT.

Industrial Applicability:
For the assessment of claims 1 to 26 on the question of whether or not they define subject matter that has industrial applicability, no unified criteria exists in the PCT. Further, the patentability of said claims can depend upon their formulation. The methods *per se* defined in claims 1 to 26 relate to subject matter which this Authority is not obliged to examine under Rule 67.1(iv) of the PCT, but the alleged effects of specific compounds referred to therein for the treatment of cancer appear to represent subject matter that has industrial applicability under Article 33(4) of the PCT.

However, claims 1 to 26 are directed to the use of antisense ODNs that are defined in such a vague and broad manner as to encompass antisense ODNs that are inoperable. D2 discloses that the nucleotide sequence of the antisense ODN targeted to TS determines its utility in treating neoplastic cells. D7 specifically discloses that ORNs to TS that are shorter than 18 nucleotides did not inhibit the expression of TS protein in human colon cancer cells. Thus, the specific nucleotide sequence and length of the ODN determines the utility of a specific ODN. It appears that the alleged effects of the specific antisense ODN depicted in SEQ ID NO:1 for the treatment of cancer represents subject matter that has industrial applicability.

Additionally, it appears that the chemotherapeutic drug must be one that targets thymidylate synthase (claims 9, 10, 18, and 19) and the cells must be resistant to the chemotherapeutic drug that targets thymidylate synthase (claims 7, 16, and 26) in order to achieve the enhanced effect of the combination of the antisense ODN and chemotherapeutic agent when compared to the use of either the antisense ODN or the chemotherapeutic agent alone. The following chemotherapeutic drugs have demonstrated industrial applicability when used in combination with the antisense ODN that is depicted in SEQ ID NO:1 of the present application: methotrexate (D2), 5-FU, 5-FUdR, and Tomudex™ (D1, D2, D3, and D4). In contrast, the following chemotherapeutic drugs did not result in an enhanced therapeutic effect when used in combination with the antisense ODN depicted in SEQ ID NO:1and thus, have no industrial applicability: cisplatin and chloroambucil (D3), melphalan, doxorubicin, and paclitaxel (D4). Thus, claim 10, which is directed to drugs including cisplatin, includes elements that have no industrial applicability. As it is apparent that not all chemotherapeutic drugs have industrial applicability, the specific chemotherapeutic drug having industrial applicability must be identified in the claims.

In view of D1-D4 and D7, claims 1-26 include subject matter that lacks industrial applicability under Article 33(4) of the PCT.